

| Final

# Acute (4-hour) inhalation toxicity study with in water' in rats

Date 3 May, 2011

Authors

Sponsor

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## Statement of GLP compliance

I, the undersigned, hereby declare that this report constitutes a complete and accurate representation of the study and its results. All study activities performed by \_\_\_\_\_ were carried out in compliance with the current OECD Principles of Good Laboratory Practice<sup>1</sup>. The OECD principles of Good Laboratory Practice are accepted by Regulatory Authorities throughout the European Community, USA and Japan. Chemical analysis for the verification of \_\_\_\_\_ in \_\_\_\_\_ water' identity and properties was not performed in this study.

**Study director**

03 - 05 - 2011  
\_\_\_\_\_  
Date (dd-mm-yyyy)

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<sup>1</sup> The most recent endorsement of compliance of the test facility with these principles is attached to the report as Annex I

## Authentication by co-operating scientists

I, the undersigned, hereby declare that the pathology data presented in this report were compiled by me or under my supervision, and accurately reflect the raw data.

\_\_\_\_\_  
Name

~~\_\_\_\_\_  
Signature~~ ✓

03-05-2011  
\_\_\_\_\_  
Date (dd-mm-yyyy)

3 May, 2011

## Quality Assurance Statement

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all audits were reported to the respective study director and management on the dates indicated.

Phase	*	Start date of audit	Date of audit report
Authorised study plan	Yes	20 December 2010	20 December 2010
Authorised study plan amendment 1	Yes	23 December 2010	23 December 2010
Authorised study plan amendment 2	Yes	28 February 2011	28 February 2011
Authorised study plan amendment 3	Yes	2 May 2011	2 May 2011
Authorised study plan amendment 4	Yes	2 May 2011	2 May 2011
Animal receipt	No	8 December 2010	8 December 2010
Animal allocation	No	22 November 2010	22 November 2010
Analytical inhalation techniques	Yes	22 December 2010	22 December 2010
Inhalatory dosing	Yes	22 December 2010	22 December 2010
Body weight	No	18 January 2011	18 January 2011
Clinical signs	No	18 January 2011	18 January 2011
Necropsy	Yes	23 December 2010	23 December 2010
Histology	No	5 January 2011	5 January 2011
Pathology	Yes	31 January 2011	31 January 2011
Pathology	Yes	4 February 2011	4 February 2011
Draft report and study file	Yes	28 March 2011	31 March 2011
Final report	Yes	2 May 2011	2 May 2011

\* Study plan, report and test substance related experimental phases are audited in a study-based manner. Other experimental phases are audited in a process-based manner. This column indicates whether or not the audit was of this particular study.

Quality assurance audits at the pathology peer review test site are presented in annex 5.

Date : 3 May 2011

Quality Assurance auditor

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## Summary

The aim of the present study was to investigate the acute inhalation toxicity of **in water** in rats. Therefore, three groups of six male and six female rats each were exposed to target concentrations of 0.1 or 1 g/m<sup>3</sup> (solid fraction) of the test material or to clean air (control group) for four hours. Three animals/sex/group were sacrificed one day after exposure; the remaining animals were sacrificed after a 14-day observation period. To characterize the toxicity, data on clinical observations, body weights, lung weights, macroscopic observations at necropsy and histopathology of the complete respiratory tract were collected.

The actual concentration, based on gravimetric analysis of the solid fraction of the test atmosphere was 0.12 and 0.97 g/m<sup>3</sup> for the low and high concentration, respectively. The mass median aerodynamic diameter (MMAD) was 3.9 and 3.4 µm (duplicate measurements) for the low concentration, and the distribution of particle sizes had a geometric standard deviation (gsd) of 1.9 and 2.3, respectively. For the high concentration, the MMAD was 3.9 and 4.1 µm, with a gsd of 2.0 and 2.1, respectively.

During exposure, the animals did not show any clinical abnormalities. In addition, no treatment-related abnormalities were observed during the 14-day observation period. Mortality did not occur during the study.

No treatment-related changes in body weight were observed. Body weight gain was as expected for animals of this strain and age.

At necropsy one day after exposure, relative lung weight was slightly – but statistically significantly – increased in male animals of the high concentration group. Absolute lung weights were unaffected in males and females one day after exposure. At the end of the 14-day observation period, no treatment-related changes in absolute and relative lung weights were observed. Taking into account that the combination of body weight and lung weight was within the historical control range, the increase in relative lung weight one day after exposure was only slight, was observed in one sex only, was reversible within a 14-day observation period, and could not be correlated to any treatment-related histopathological changes, it seems unlikely that the elevated relative lung weight in males one day after exposure constitutes a toxicologically significant effect.

No treatment-related gross abnormalities were found during macroscopic examination at necropsy. Moreover, microscopic examination of the respiratory tract did not reveal any exposure-related histopathological changes.

### Conclusion

The only treatment-related effect in response to the 4-hour inhalation exposure to **in water** was a slightly increased relative lung weight in male animals one day after exposure. However, it seems unlikely that this constitutes a toxicologically significant effect. Therefore, the No-Observed-Adverse-Effect-Level (NOAEL) for acute (4-hour) inhalation toxicity was considered to be 0.97 g/m<sup>3</sup> (solid fraction), the highest concentration tested.

# 1 General

## 1.1 Study sponsor and monitor

Sponsor:

Monitor:

Telephone:

E-mail:

## 1.2 Test facility

Postal address:

Location:

Telephone:

Telefax:

As of 1 January 2011 the major part of the Business Unit Quality and Safety of  
has continued as a wholly-owned subsidiary of

## 1.3 Test site

An external peer review of the pathology was performed by the sponsor.

## 1.4 Responsible personnel

Study director:

Management:

Scientific contributor:

Principal investigator:

<sup>1</sup> Department of Toxicology and Applied Pharmacology.

<sup>2</sup> E-mail: Phone Fax

<sup>3</sup> Responsible for external peer review of the pathology, performed by the sponsor

## 1.5 Time schedule

Arrival of the animals:	15 December 2010
Exposure of the animals:	22 December 2010
Sacrifice of the animals (first groups):	23 December 2010
Sacrifice of the animals (second groups):	5 January 2011

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## 2 Introduction

### 2.1 Objective

At the request of  
an acute (4-hour) inhalation toxicity study with  
**in water** was carried out in rats.

Data on clinical observations, body weights, lung weights, macroscopic observations at necropsy and histopathology of the complete respiratory tract were used as criteria for disclosing possible harmful non-lethal effects.

### 2.2 Applicable guidelines

The study was conducted - in as far as applicable - in accordance with the following guideline:

- OECD Guideline for the Testing of Chemicals 403, acute inhalation toxicity study, adopted 7 September 2009

### 2.3 Animal welfare

The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 86/609/EEC) and Dutch legislation (The Experiments on Animals Act, 1997). This included approval of the study by ethical review committee ( ).

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### 3 Study plan and deviations

#### 3.1 Study Plan

The study was conducted according to study plan entitled: "Acute (4-hour) inhalation toxicity study with in water' in rats" and 4 amendments. The study plan was approved by the study director on 16 December 2010.

#### 3.2 Deviations

At the time of exposure, the animals were 7 weeks of age, instead of 8-12 weeks as mentioned in the study plan.

In the period 18-23 December 2010, the temperature in the animal room was frequently below 20°C for short periods of time, with a minimum temperature of 19.8°C. Relative humidity was above 65% on 29 December 2010 for approximately 30 minutes (maximum of 66.7%) and below 45% on 4 January 2011 for approximately 20 minutes (minimum of 40.7%).

Inadvertently, statistical analysis was performed on body and lung weight data (see paragraph 4.5.8) as part of the standard procedures, although this was not mentioned in the study plan.

Deviations from the study plan concerning the work performed at the test site are described in Annex 5.

These deviations are considered not to have affected the validity of the study.

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## 4 Materials and methods

### 4.1 Test material

The test material was supplied by the sponsor in a single container, labeled  
 ; Container No. 1; exp Dec. 1, 2011;  
 Storage: NRT&H; gross wt 2,404.9 g; net wt 2,006.4 g; Date 10-18-10'. The container  
 was received in good condition on 26 November 2010. The test material was stored in a  
 chemical safety cabinet at ambient temperature. The  
 was

**in water** is an amber liquid and has the  
 following characteristics (as given by the sponsor):

Name	:	in water
Other names	:	
Chemical name	:	

Composition of carrier	:	
Total quantity	:	2 L
Batch / Lot number	:	
Purity	:	
pH	:	
Volatile	:	no
Storage conditions	:	ambient temperature
Expiry date	:	1 December 2011

Any analysis for the identity, quality and purity of the test material, with supporting  
 documentation was the responsibility of the sponsor (see Annex 2).

### 4.2 Test system

#### 4.2.1 *Animal characterization*

Adult, male and female Wistar rats (CrI:WI[WU], outbred) were obtained from a  
 colony maintained under SPF-conditions by Charles River Laboratories.

On 15 December 2010, 20 male and 20 female animals arrived at an age of 6 weeks.  
 They were taken in their unopened shipping containers to animal room 5.1.15, were  
 checked for overt signs of ill health and anomalies, and were kept in quarantine. After  
 approval of the lot (negative titers to micro-organisms tested in a few animals),  
 quarantine was raised on 17 December 2010 and the animals were moved to room  
 6.0.06, a similar animal room. The rats were separated by sex and uniquely identified  
 by ear tattoo.

Just before the start of the study on 21 December 2010, the animals were randomly  
 allocated to the different groups, taking their body weight into account. The average

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body weights of the rats on day 0 before exposure were 208.1 g and 146.6 g for the males and females, respectively. The duration of the acclimatization period was 5 days.

#### 4.2.2 *Animal identification*

The study was identified as study number                      After allocation to the study, a number and a color coded each group of rats. The individual rats were identified by unique animal identification numbers which were tattooed in their ears (see Annex 3). Each cage was provided with a card showing the study number, color code, group number, cage number and animal identification numbers.

### 4.3 **Experimental conditions**

#### 4.3.1 *Animal maintenance*

The animals were housed under conventional conditions in macrolon cages with bedding of wood shavings (Lignocel, type <sup>3</sup>/<sub>4</sub>, Rettenmaier, Rosenberg, Germany) and strips of paper (Enviro-dri, Lillico, Betchworth, England) as environmental enrichment. The number of air changes was about 10 per hour. Before allocation to the different groups, the animals were housed 5 males or 5 females to a cage. During the study, the animals were housed 3 males or 3 females to a cage. During the exposure, the animals had no access to feed or water and were housed individually in the holders. After exposure, the animals returned to their living cages and were held for an observation period of 1 or 14 days before sacrifice and necropsy.

The temperature in the animal room was within the range of 20 – 24°C and relative humidity was within the range of 45 - 65%, except during short periods associated with cleaning activities. In the period 18-23 December 2010, the temperature was frequently below 20°C for short periods of time, with a minimum temperature of 19.8°C. Relative humidity was above 65% on 29 December 2010 for approximately 30 minutes (maximum of 66.7%) and below 45% on 4 January 2011 for approximately 20 minutes (minimum of 40.7%). A 12-hour light and 12-hour dark cycle was maintained.

#### 4.3.2 *Feed and drinking water*

Feed and drinking water were provided *ad libitum* from the arrival of the animals until the end of the study, except during exposure. All rats were fed a commercially available rodent diet (Rat & Mouse No. 3 Breeding Diet RM3). Each batch of this diet is analysed for nutrients and contaminants by the supplier, SDS Special Diet Services, Whitham, England. The certificate of analysis pertaining to the batches used (Batch nos. 7758 and 7988) will be kept in the archives and will be available upon request.

Each cage was supplied with domestic mains tap-water suitable for human consumption (quality guidelines according to Dutch legislation based on EC Council Directive 98/83/EC). The water was given in polypropylene bottles, which were cleaned weekly and filled as needed. Results of the routine physical, chemical and microbial examination of the drinking water as conducted by the supplier are made available to the test facility. In addition, the supplier periodically (twice per year) analyses water samples taken on the premises for a limited number of variables. The results of these analyses will be kept in the archives and will be available upon request.

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#### 4.4 Experimental procedures

##### 4.4.1 Administration of the test material

The test material was administered to the animals by inhalation. This route of administration was chosen because humans may be exposed to the test material by inhalation. The control group was exposed to clean air, but otherwise treated in the same manner as the exposed groups.

##### 4.4.2 Number and size of test groups, exposure levels and acclimatization

The study started with the exposure of the animals on 22 December 2010 (day 0). The study comprised three test groups of 6 male and 6 female rats each, viz. one control group exposed to humidified clean air and two groups exposed to different concentrations of the test material for a single 4-hour period. Three animals/sex/group were sacrificed one day after exposure on 23 December 2010. The remaining animals were kept for an observation period of 14 days before sacrifice on 5 January 2011. Before the start of the study, the animals were adapted to the restraint by placing them in Battelle restraining tubes for approximately 10 minutes/day on days -1, -2 and -3.

The various groups are presented in the table below.

Group No.	Group description	Colour code	Target concentration in air (g/m <sup>3</sup> solids)	Number of animals/group (sacrifice on day 1)	Number of animals/group (sacrifice on day 14)
1	Control	White	0	3 ♂ and 3 ♀	3 ♂ and 3 ♀
2	Low	Blue	0.1	3 ♂ and 3 ♀	3 ♂ and 3 ♀
3	High	Green	1.0	3 ♂ and 3 ♀	3 ♂ and 3 ♀

##### 4.4.3 Exposure equipment

The animals were exposed to the test atmosphere in nose-only inhalation chambers (a modification of the design of the chamber manufactured by ADG Developments Ltd., Codicote, Hitchin, Herts, SG4 8UB, United Kingdom; see Figure 1). The inhalation chambers consisted of a cylindrical polypropylene (group 1) or stainless steel (groups 2 and 3) column, surrounded by a transparent cylinder. The columns had a volume of ca. 60 litres and consisted of a top assembly with the entrance of the unit, two or three mixing chambers, one or two rodent tube section, and the exhaust section at the bottom. The rodent tube sections had 20 ports for animal exposure. Several empty ports were used for test atmosphere sampling, particle size analysis, measurement of oxygen concentration, temperature and relative humidity. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. Male and female rats were placed in alternating order. The remaining ports were closed. Only the nose of the rats protruded into the interior of the column.

In our experience, the animal's body does not exactly fit in the animal holder which always results in some leakage from high to low pressure side. By securing a positive pressure in the central column and a slightly negative pressure in the outer cylinder, which encloses the entire animal holder, air leaks from nose to thorax rather than from

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thorax to nose and dilution of the test atmosphere at the nose of the animals is prevented.

#### 4.4.4 *Generation of the test atmosphere*

The inhalation equipment was designed to expose rats to a continuous supply of fresh test atmosphere. The test material was diluted with water (mass-based dilution factor of 1.4) to reduce the particle size. To generate the test atmosphere, the diluted test material was passed via a motor driven syringe pump (WPI Type SP220i, World Precision Instruments, Sarasota FL, USA) to a pressurized air atomizer (Schlick type 970/S, Coburg, Germany). The atomizer was placed at the top inlet of the exposure unit and supplied with humidified (group 2 only), pressurized air, the flow of which was measured by a mass stream meter (Bronkhorst Hi Tec, The Netherlands). The settings of the pumps and the air pressure on the atomizers were recorded at regular intervals (approximately each half hour) during the generation of the test atmosphere. The readings of the mass stream meters were recorded continuously using an analogue chart recorder (Kipp & Zonen, Delft, The Netherlands). The control atmosphere (group 1) consisted of a mass flow controlled (Bronkhorst Hi Tec) stream of humidified compressed air.

From the top inlet of the exposure unit, the resulting test atmosphere was directed downward through the mixing chambers towards the animals. At the bottom of the unit the test atmosphere was exhausted (see Figure 1). The airflow through the exposure chambers during exposure was 27 L/min. The animals were placed in the exposure unit after stabilization of the test atmosphere. The period between the start of the generation of the test atmosphere and the start of exposure of the animals was 49 (group 2) or 51 (group 3) minutes. The concentration  $C$  in a perfectly stirred test atmosphere in a chamber with volume  $V$  (L) and flow  $F$  (L/min) increases according to  $C = C_{\infty} * (1 - e^{-(F*T/V)})$ , in which  $T$  (min) is the time and  $C_{\infty}$  is the steady state concentration. Hence  $T_{95}$ , the time it takes to reach 95% of the steady state concentration is given by  $e^{-(F*T_{95}/V)} = 0.05$ , from which it follows that  $T_{95}$  was approximately 7 minutes. In practice, after the start of the generation the aerosol will spread from the top to the bottom and  $T_{95}$  will be shorter than in a perfectly stirred chamber.

### 4.5 **Observations, analyses and measurements**

#### 4.5.1 *Actual concentration*

The concentration of the solid fraction of the aerosol present in the test atmosphere was determined four times during exposure (approximately once each hour) by means of gravimetric analysis. Representative test atmosphere samples were obtained by passing approximately 5 L (group 3) or 50 L (group 2) test atmosphere at 5 L/min through fibre glass filters (Whatman, GF10, Ø 47 mm). Filters were weighed before sampling, loaded with a sample of test atmosphere, and weighed again. During preliminary measurements, it was established that drying of the loaded filters was not necessary, because the weight was constant.

#### 4.5.2 *Nominal concentration*

The nominal concentration of the solid fraction was determined by dividing the total amount of test material used (by weighing) by the total volume of air passed through the exposure unit, taking the initial water content of the test material and the dilution factor into account.

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#### 4.5.3 *Particle size measurement*

Three particle size distribution measurements were carried out for groups 2 and 3, one during preliminary generation of the test atmosphere and two during exposure. A 10-stage cascade impactor was used (Sierra 210, model 2110K, Graseby Andersen, Smyrna, GA, USA). The Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (gsd) were calculated (Lee, 1972).

#### 4.5.4 *Temperature, relative humidity and oxygen content*

The temperature and the relative humidity of the test atmosphere was recorded eight times during exposure at regular intervals (about twice per hour) using an RH/T device (TESTO 635, TESTO GmbH & Co, Lenzkirch, Schwarzwald, Germany). The oxygen concentration was checked once during exposure (Oxygen analyser type PMA-10, M&C Products Analysentechnik GmbH, Ratingen-Lintorf, Germany).

#### 4.5.5 *Clinical signs*

The rats were visually inspected just before exposure, for reactions to treatment during the exposure, shortly after exposure, and at least once daily during the observation period.

#### 4.5.6 *Body weights*

The body weight of each animal was recorded one day before exposure (day -1), just prior to exposure on day 0, and on day 1. The body weights of the rats that were held for a 14-day observation period were recorded on days 3, 7 and 14 as well.

#### 4.5.7 *Pathology*

##### Macroscopic examination

At the end of the observation period (1 day after exposure for 3 animals/sex/group and 14 days after exposure for the remaining 3 animals/sex/group), animals were killed in such a sequence that the average time of killing was approximately the same for each group. The animals were killed by exsanguination from the abdominal aorta under pentobarbital anaesthesia and then examined grossly for pathological changes.

The lungs with trachea and larynx were removed and weighed. For histopathological examination, the complete respiratory tract including nasopharyngeal tissues and all relevant gross lesions were preserved in a neutral aqueous phosphate-buffered 4 per cent solution of formaldehyde (10% solution of formalin). The lungs (after weighing) were infused with the fixative under ca. 15 cm water pressure to insure fixation.

##### Slide preparation

Tissues to be examined were embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin.

##### Histopathological examination

All preserved tissues of all animals of the control and high concentration groups sacrificed on day 1 and 14 were examined histopathologically (by light microscopy). In addition, all relevant gross lesions observed in rats of the low concentration group were examined microscopically. The nasopharyngeal tissues were examined at 6 levels (Woutersen et al., 1994; Annex 4), the larynx at 3 levels (including the base of the epiglottis), the trachea at 3 levels (including a longitudinal section through the carina of the bifurcation), and each lung lobe at 1 level.

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After completion of the histopathological examination at the test facility (including an internal peer review according to standard procedures), the microscopic slides were sent to the sponsor for an external peer review of the pathology (see Annex 5).

#### 4.5.8 *Statistical analysis of the results*

Body and lung weight data were analysed using one-way analysis of variance (Anova), after checking for homogeneity of variance (Levene's test) and normality of data distribution (Shapiro-Wilks test). If variances were not homogeneous or data not normally distributed, the data were stepwise log or rank transformed prior to the Anova. If the Anova yielded a significant effect ( $p < 0.05$ ), intergroup comparisons with the control group were made by Dunnett's multiple comparison test.

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## 5 Results

### 5.1.1 *Actual concentration*

The actual concentrations ( $\pm$  standard deviation, number of measurements), based on gravimetric analysis of the solid fraction of the aerosol in the test atmosphere, were  $0.12 \text{ g/m}^3$  ( $\pm 0.03$ ,  $n=4$ ; Table 1.1.1) and  $0.97 \text{ g/m}^3$  ( $\pm 0.21$ ,  $n=4$ ; Table 1.1.2) for the low and high concentration, respectively.

### 5.1.2 *Nominal concentration*

Nominal concentrations, calculated from the total amount of test material used (solid fraction) and the air flow were  $0.98 \text{ g/m}^3$  for the low concentration and  $11.2 \text{ g/m}^3$  for the high concentration test atmosphere, indicating generation efficiencies of 83% and 57%, respectively.

### 5.1.3 *Particle size measurement*

During exposure, more than 85% (low concentration) or 79% (high concentration) of the mass of the aerosol present at the animals' breathing zone was contained in particles with an aerodynamic diameter smaller than  $5.7 \mu\text{m}$ . The mass median aerodynamic diameter (MMAD) was 3.9 and  $3.4 \mu\text{m}$  (duplicate measurements) for the low concentration test atmosphere and the distribution of particle sizes had a geometric standard deviation (gsd) of 1.9 and 2.3, respectively. For the high concentration atmosphere, the MMAD was determined to be 3.9 and  $4.1 \mu\text{m}$ , with a gsd of 2.0 and 2.1, respectively. During preliminary generation of both test atmospheres, using the same settings, approximately the same values were found (Tables 1.2.1 – 1.2.6).

### 5.1.4 *Temperature, relative humidity and oxygen content*

The mean temperature ( $\pm$  standard deviation) during exposure was  $21.9 (\pm 0.4)$ ,  $21.6 (\pm 0.4)$  and  $21.3 (\pm 0.3) ^\circ\text{C}$  for the control, low and high concentration, respectively. Mean relative humidity ( $\pm$  standard deviation) was  $54 (\pm 3)$ ,  $48 (\pm 3)$  and  $54 (\pm 2) \%$  for the control, low and high concentration, respectively (Tables 1.3.1 – 1.3.3). The oxygen concentration, measured during the exposure in the exposure chambers was 20.6% for all three test atmospheres.

### 5.1.5 *Clinical signs*

The animals did not demonstrate any clinical abnormalities during exposure (Tables 2.1.1 – 2.1.3). In addition, no treatment-related abnormalities were observed during the 14-day observation period (Tables 2.2.1 and 2.2.2). The nasal encrustations, which were observed on day 2 in a single male animal of group 3, were considered not to be related to the exposure. Mortality did not occur during the study.

### 5.1.6 *Body weights*

No differences in body weight were observed between the groups (Tables 3.1 and 3.2). Body weight gain of all animals was as expected for animals of this strain and age.

### 5.1.7 *Lung weights*

At necropsy one day after exposure, relative lung weights were slightly – but statistically significantly – increased in males of the high concentration group, when compared to unexposed control animals. No changes in absolute weight of the lungs were observed in males or females one day after exposure (Tables 4.1.1 and 4.1.2).

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No differences in absolute or relative lung weight were observed between the groups at the end of the 14-day recovery period for males and females (Tables 4.2.1 and 4.2.2).

#### 5.1.8 *Pathology*

Macroscopic examination (Tables 5.1 and 5.2, Appendix 1)

Macroscopic examination did not reveal any treatment-related gross changes.

Microscopic examination (Tables 6.1 and 6.2, Appendix 1)

Microscopic examination did not reveal any treatment-related histopathological changes.

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## 6 Discussion and conclusion

The aim of the present study was to investigate the acute inhalation toxicity of **in water** in rats. Three groups of six male and six female rats each were exposed to concentrations of 0.12 or 0.97 g/m<sup>3</sup> of the test material (based on gravimetric analysis of the solid fraction) or to clean air (control group) for four hours. The particle size (MMAD) of the particles in the aerosol was 3.9 and 3.4 µm (duplicate measurements) for the low concentration, and the distribution of particle sizes had a geometric standard deviation (gsd) of 1.9 and 2.3, respectively. For the high concentration aerosol, the MMAD was 3.9 and 4.1 µm, with a gsd of 2.0 and 2.1, respectively. Three animals/sex/group were sacrificed one day after exposure; the remaining animals were sacrificed after a 14-day observation period.

The animals did not show any treatment-related clinical abnormalities during exposure or during the 14-day observation period. In addition, no effects on body weight were observed in response to exposure to the test substance. Mortality did not occur during the study. No treatment-related gross abnormalities were found during macroscopic examination at necropsy. Moreover, microscopic examination of the respiratory tract did not reveal any exposure-related histopathological changes. At necropsy one day after exposure, relative lung weight was slightly – but statistically significantly – increased in male animals of the high concentration group. However, the combination of body weight and lung weight of these animals was within the historical control range. Absolute lung weights were unaffected in males and females one day after exposure. At the end of the 14-day observation period, no treatment-related changes in absolute and relative lung weights were observed. Taking into account that the combination of body weight and lung weight was within the historical control range, the increase in relative lung weight one day after exposure was only slight, was observed in one sex only, was reversible within a 14-day observation period, and could not be correlated to any treatment-related histopathological changes, it seems unlikely that the elevated relative lung weight in males one day after exposure constitutes a toxicologically significant effect.

### Conclusion

The only treatment-related effect in response to the 4-hour inhalation exposure to **in water** was a slightly increased relative lung weight in male animals one day after exposure. However, it seems unlikely that this constitutes a toxicologically significant effect. Therefore, the No-Observed-Adverse-Effect-Level (NOAEL) for acute (4-hour) inhalation toxicity was considered to be 0.97 g/m<sup>3</sup> (solid fraction), the highest concentration tested.

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## 7 Documentation and retention of records

The following documents and materials will be retained for 5 years:

- Raw data or true copies of these
- Correspondence
- All other information related to the study
- Tissue specimens and paraffin blocks

At the end of the retention period, the sponsor will be asked whether these documents and materials should be discarded, retained for an additional period, or transferred to the archives of the sponsor.

Master copies of the approved study plan, any amendments thereof and the final report will be retained for at least 15 years.

Remaining test substance will be retained for at least one month and then discarded. The carcass was discarded after completion of the histopathological examination. Microscopic slides will be retained for at least 15 years and then removed from the archives.

Documents and materials will be retained in the archives of , U  
The archiving period starts on the cover date of the final report.

After completion of the histopathological examination at the test facility, microscopic slides were sent to the sponsor for external peer review. After completion of the external peer review of the pathology, the slides were returned to the test facility. All raw data concerning the external peer review of the pathology will be retained in the archives of the sponsor.

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## 8 Reference

Lee R.J. Jr. The size of suspended particulate matter in air. *Science*, 178:567-575 (1972)

Woutersen R.A., van Garderen-Hoetmer A., Slootweg P.J., Feron V.J. (1994). Upper respiratory tract carcinogenesis in experimental animals and in humans. In: *Carcinogenesis*, Waalkes MP and Ward JM (eds), Target Organ Toxicology Series, Raven Press, New York, 215-263

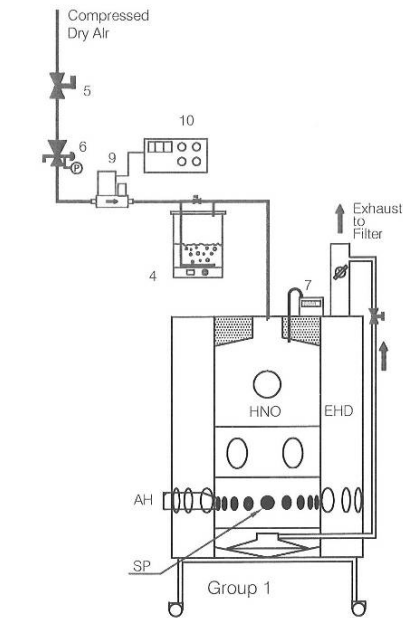
## Figures

3 May, 2011

# Acute (4-hour) inhalation toxicity study with in water' in rats

Study:

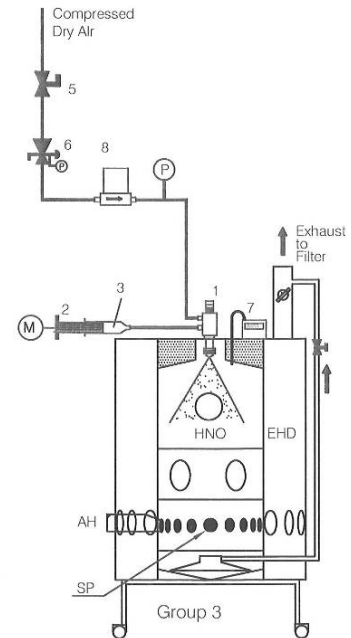
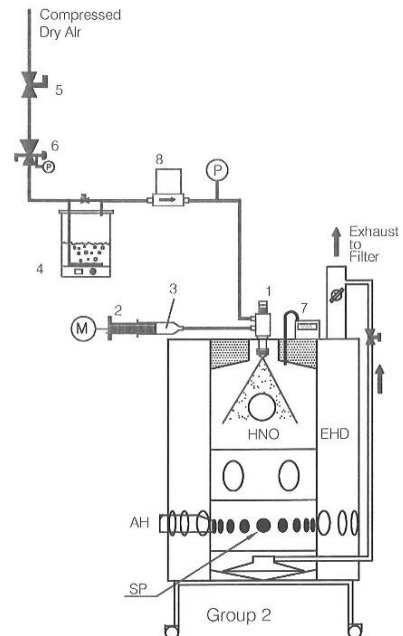
Figure 1 – Schematic diagram of the generation and exposure system



Study: 9193

- 1: Schlick Nebuliser
- 2: Motordriven Syringe Pump
- 3: Testsubstance
- 4: Humidifier
- 5: Main Valve
- 6: Reducing Valve
- 7: Pressure Gauge
- 8: MassStreamMeter
- 9: Mass Flow Controller
- 10: Control Unit MFC

HNO: Head/Nose-Only Unit  
 EHD: External Hood  
 AH: Animal Holder  
 SP: Sample Port  
 Measurement O<sub>2</sub> & T/RH, Particle  
 Size & Gravimetric Analysis  
 P: Manometer



## Tables

3 May, 2011

Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.1.1 - Actual concentration of the solid fraction of the test material in the test atmosphere as measured by gravimetric analysis – group 2

Sample time (hh:mm)	Volume sampled (L)	Mass sampled (mg)	Aerosol concentration (g/m <sup>3</sup> )
10:05	50.2	4.93	0.098
11:06	50.2	8.68	0.173
11:31	50.2	5.52	0.110
12:26	50.2	5.61	0.112
mean			0.123
sd			0.034

Generation of the test atmosphere: 8:24 – 13:18

Animals were exposed in the period: 9:13 – 13:13

Table 1.1.2 - Actual concentration of the solid fraction of the test material in the test atmosphere as measured by gravimetric analysis – group 3

Sample time (hh:mm)	Volume sampled (L)	Mass sampled (mg)	Aerosol concentration (g/m <sup>3</sup> )
9:33	5.02	3.75	0.747
10:30	5.02	4.23	0.843
11:33	5.02	5.70	1.135
12:30	5.02	5.84	1.163
mean			0.972
sd			0.209

Generation of the test atmosphere: 8:24 – 13:18

Animals were exposed in the period: 9:15 – 13:15

3 May, 2011

Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.1 – Aerodynamic particle size distribution in the test atmosphere during preliminary generation of the test atmosphere on 21 December 2010 – group 2

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.02	35.1	98.9
2	0.03	23.7	97.3
3	0.06	9.0	94.0
4	0.23	5.7	81.4
5	0.43	4.5	57.9
6	0.61	1.7	24.6
7	0.14	1.4	16.9
8	0.13	0.7	9.8
9	0.10	0.6	4.4
10	0.03	0.3	2.7
Back up filter	0.05		
Total (mg)	1.83		
Volume (l)	20.72		
Flow (l/min)	2.07		
MMAD	3.9		
gsd	2.2		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.2 – Aerodynamic particle size distribution in the test atmosphere during exposure of the animals, sampled at approximately 9:45 – group 2

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.00	35.1	100.0
2	0.00	23.8	100.0
3	0.00	9.0	100.0
4	0.23	5.7	85.8
5	0.44	4.5	58.6
6	0.59	1.7	22.2
7	0.13	1.4	14.2
8	0.14	0.7	5.6
9	0.08	0.6	0.6
10	0.01	0.3	0.0
Back up filter	0.00		
Total (mg)	1.62		
Volume (l)	20.70		
Flow (l/min)	2.07		
MMAD	3.9		
gsd	1.9		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.3 – Aerodynamic particle size distribution in the test atmosphere during exposure of the animals, sampled at 11:46 – group 2

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.00	35.1	100.0
2	0.00	23.8	100.0
3	0.01	9.0	99.4
4	0.21	5.7	87.4
5	0.43	4.5	62.9
6	0.58	1.7	29.7
7	0.15	1.4	21.1
8	0.15	0.7	12.6
9	0.10	0.6	6.9
10	0.07	0.3	2.9
Back up filter	0.05		
Total (mg)	1.75		
Volume (l)	20.70		
Flow (l/min)	2.07		
MMAD	3.4		
gsd	2.3		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4- inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.4 – Aerodynamic particle size distribution in the test atmosphere during preliminary generation of the test atmosphere on 20 December 2010 – group 3

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.00	35.0	100.0
2	0.00	23.7	100.0
3	0.00	9.0	100.0
4	0.23	5.7	85.8
5	0.42	4.5	59.9
6	0.58	1.7	24.1
7	0.15	1.4	14.8
8	0.17	0.7	4.3
9	0.06	0.6	0.6
10	0.01	0.3	0.0
Back up filter	0.00		
Total (mg)	1.62		
Volume (l)	2.08		
Flow (l/min)	2.08		
MMAD	3.7		
gsd	2.0		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.5 – Aerodynamic particle size distribution in the test atmosphere during exposure of the animals, sampled at 10:23 – group 3

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.00	35.1	100.0
2	0.00	23.8	100.0
3	0.00	9.0	100.0
4	0.24	5.7	82.9
5	0.35	4.5	57.9
6	0.49	1.7	22.9
7	0.12	1.4	14.3
8	0.13	0.7	5.0
9	0.04	0.6	2.1
10	0.00	0.3	2.1
Back up filter	0.03		
Total (mg)	1.4		
Volume (l)	2.07		
Flow (l/min)	2.07		
MMAD	3.9		
gsd	2.0		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.2.6 – Aerodynamic particle size distribution in the test atmosphere during exposure of the animals, sampled at 12:05 – group 3

Stage	Captured mass (mg)	Cut-off size ( $\mu\text{m}$ )	% of mass smaller than cut-off
1	0.00	35.1	100.0
2	0.00	23.8	100.0
3	0.00	9.0	100.0
4	0.38	5.7	79.8
5	0.47	4.5	54.8
6	0.62	1.7	21.8
7	0.16	1.4	13.3
8	0.16	0.7	4.8
9	0.07	0.6	1.1
10	0.00	0.3	1.1
Back up filter	0.02		
Total (mg)	1.88		
Volume (l)	2.07		
Flow (l/min)	2.07		
MMAD	4.1		
gsd	2.1		

MMAD = Mass Median Aerodynamic Diameter.

gsd = mean geometric standard deviation (square root (84<sup>th</sup> percentile of the cumulative distribution) / (16<sup>th</sup> percentile of the cumulative distribution)).

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.3.1 – Temperature and relative humidity during exposure – group 1

Time of measurement	T (°C)	RH (%)
9:24	21.2	58.3
9:55	21.5	55.5
10:25	21.8	53.0
10:55	22.0	51.8
11:25	21.8	50.8
11:55	22.1	49.9
12:22	22.0	53.3
12:52	22.4	59.0
average	21.9	54.0
sd	0.4	3.4
n	8	8
max	22.4	59.0
min	21.2	49.9

Animals were exposed in the period: 9:11 – 13:11

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.3.2 – Temperature and relative humidity during exposure – group 2

Time of measurement	T (°C)	RH (%)
9:34	20.9	52.0
10:05	21.3	47.3
10:35	21.4	46.3
11:05	21.6	44.4
11:35	21.8	47.6
12:05	21.8	47.1
12:32	21.7	48.4
12:57	22.0	51.8
average	21.6	48.1
sd	0.4	2.6
n	8	8
max	22.0	52.0
min	20.9	44.4

Animals were exposed in the period: 9:13 – 13:13

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 1.3.3 – Temperature and relative humidity during exposure – group 3

Time of measurement	T (°C)	RH (%)
9:44	20.8	51.2
10:10	21.2	50.7
10:45	21.4	54.7
11:15	21.5	53.7
11:45	21.6	52.9
12:12	21.2	55.1
12:42	21.0	55.9
13:10	21.6	55.0
average	21.3	53.7
sd	0.3	1.9
n	8	8
max	21.6	55.9
min	20.8	50.7

Animals were exposed in the period: 9:15 – 13:15

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 2.1.1 – Clinical signs during exposure – group 1

Animal number	Clinical signs	Time of observation
Male animals		
2, 4, 6, 8, 10, 12	No abnormalities	10:11; 11:11; 12:10; 13:08
Female animals		
1, 3, 5, 7, 9, 11	No abnormalities	10:11; 11:11; 12:10; 13:08

Animals were exposed in the period: 9:11 – 13:11

Table 2.1.2 – Clinical signs during exposure – group 2

Animal number	Clinical signs	Time of observation
Male animals		
14, 16, 18, 20, 22, 24	No abnormalities	10:13; 11:13; 12:12; 13:09
Female animals		
13, 15, 17, 19, 21, 23	No abnormalities	10:13; 11:13; 12:12; 13:09

Animals were exposed in the period: 9:13 – 13:13

Table 2.1.3 – Clinical signs during exposure – group 3

Animal number	Clinical signs	Time of observation
Male animals		
26, 28, 30, 32, 34, 36	No abnormalities	10:15; 11:15; 12:15; 13:10
Female animals		
25, 27, 29, 31, 33, 35	No abnormalities	10:15; 11:15; 12:15; 13:10

Animals were exposed in the period: 9:15 – 13:15

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 2.2.1 – Clinical signs after exposure and during the observation period – Males

Day numbers during which observation was seen (relative to Start Date)

Group	Sex	Animal	Clinical Sign	
-----				
1	m	2	DEAD Killed scheduled	( 1 - 1 )
		4	DEAD Killed scheduled	( 1 - 1 )
		6	DEAD Killed scheduled	( 1 - 1 )
		8	DEAD Killed scheduled	( 14 - 14 )
		10	DEAD Killed scheduled	( 14 - 14 )
		12	DEAD Killed scheduled	( 14 - 14 )
2	m	14	DEAD Killed scheduled	( 1 - 1 )
		16	DEAD Killed scheduled	( 1 - 1 )
		18	DEAD Killed scheduled	( 1 - 1 )
		20	DEAD Killed scheduled	( 14 - 14 )
		22	DEAD Killed scheduled	( 14 - 14 )
		24	DEAD Killed scheduled	( 14 - 14 )
3	m	26	DEAD Killed scheduled	( 1 - 1 )
		28	DEAD Killed scheduled	( 1 - 1 )
		30	DEAD Killed scheduled	( 1 - 1 )
		32	DEAD Killed scheduled	( 14 - 14 )
			NOSE Encrustation(s)	( 2 - 2 )
		34	DEAD Killed scheduled	( 14 - 14 )
		36	DEAD Killed scheduled	( 14 - 14 )

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 2.2.2 – Clinical signs after exposure and during the observation period – Females

Day numbers during which observation was seen (relative to Start Date)

Group	Sex	Animal	Clinical Sign	
-----				
1	f	1	DEAD Killed scheduled	( 1 - 1 )
		3	DEAD Killed scheduled	( 1 - 1 )
		5	DEAD Killed scheduled	( 1 - 1 )
		7	DEAD Killed scheduled	( 14 - 14 )
		9	DEAD Killed scheduled	( 14 - 14 )
		11	DEAD Killed scheduled	( 14 - 14 )
2	f	13	DEAD Killed scheduled	( 1 - 1 )
		15	DEAD Killed scheduled	( 1 - 1 )
		17	DEAD Killed scheduled	( 1 - 1 )
		19	DEAD Killed scheduled	( 14 - 14 )
		21	DEAD Killed scheduled	( 14 - 14 )
		23	DEAD Killed scheduled	( 14 - 14 )
3	f	25	DEAD Killed scheduled	( 1 - 1 )
		27	DEAD Killed scheduled	( 1 - 1 )
		29	DEAD Killed scheduled	( 1 - 1 )
		31	DEAD Killed scheduled	( 14 - 14 )
		33	DEAD Killed scheduled	( 14 - 14 )
		35	DEAD Killed scheduled	( 14 - 14 )

Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 3.1 – Individual and mean body weight - Males

Group	Sex	Animal	Body weight (Gram)					
			-----					
			Day numbers relative to Start Date					
			-1	0	1	3	7	14
1	m	2	205.2	210.4	212.9	.	.	.
		4	196.0	200.9	201.1	.	.	.
		6	207.5	214.3	213.2	.	.	.
		8	219.5	224.1	225.8	233.1	263.9	301.4
		10	202.0	206.4	207.8	216.8	241.3	277.6
		12	197.9	201.2	203.6	213.6	235.0	261.6
		-----	-----	-----	-----	-----	-----	-----
		Mean	204.68	209.55	210.73	221.17	246.73	280.20
		S.D.	8.44	8.83	8.83	10.46	15.20	20.03
2	m	N	6	6	6	3	3	3
		14	191.1	196.4	200.2	.	.	.
		16	209.0	213.3	216.3	.	.	.
		18	199.7	207.7	208.4	.	.	.
		20	202.3	203.0	208.7	217.9	246.1	279.1
		22	205.4	208.9	210.6	223.4	255.1	292.9
		24	201.8	205.0	206.8	219.4	251.1	281.8
		-----	-----	-----	-----	-----	-----	-----
		Mean	201.55	205.72	208.50	220.23	250.77	284.60
3	m	S.D.	6.06	5.77	5.24	2.84	4.51	7.31
		N	6	6	6	3	3	3
		26	202.0	210.5	214.7	.	.	.
		28	199.3	207.7	207.7	.	.	.
		30	207.8	213.6	215.5	.	.	.
		32	205.6	209.5	210.3	219.3	249.7	284.6
		34	204.0	208.4	211.2	221.9	248.7	281.9
		36	197.3	204.4	206.7	218.1	247.4	289.7
		-----	-----	-----	-----	-----	-----	-----
		Mean	202.67	209.02	211.02	219.77	248.60	285.40
		S.D.	3.93	3.06	3.57	1.94	1.15	3.96
		N	6	6	6	3	3	3

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 3.2 – Individual and mean body weight - Females

			Body weight (Gram)					
			-----					
Group	Sex	Animal	Day numbers relative to Start Date					
			-1	0	1	3	7	14
-----								
1	f	1	144.8	148.5	148.4	.	.	.
		3	145.7	143.7	147.8	.	.	.
		5	148.9	148.1	150.6	.	.	.
		7	142.2	144.6	145.0	147.1	160.3	168.8
		9	150.1	153.8	154.6	156.8	166.1	176.0
		11	146.6	148.1	148.8	154.0	170.0	178.2
		-----	-----	-----	-----	-----	-----	-----
		Mean	146.38	147.80	149.20	152.63	165.47	174.33
		S.D.	2.85	3.57	3.21	4.99	4.88	4.92
N	6	6	6	3	3	3		
-----								
2	f	13	149.5	146.3	154.9	.	.	.
		15	145.2	141.7	147.4	.	.	.
		17	141.8	141.8	147.0	.	.	.
		19	147.6	147.9	152.6	157.7	173.4	179.2
		21	151.7	154.8	154.8	164.5	178.3	182.5
		23	145.9	146.9	146.6	154.9	166.1	176.1
		-----	-----	-----	-----	-----	-----	-----
		Mean	146.95	146.57	150.55	159.03	172.60	179.27
		S.D.	3.47	4.82	3.98	4.94	6.14	3.20
N	6	6	6	3	3	3		
-----								
3	f	25	142.2	143.0	144.9	.	.	.
		27	150.0	151.3	150.8	.	.	.
		29	142.3	137.0	145.0	.	.	.
		31	145.4	144.5	144.6	154.6	169.6	181.6
		33	146.8	147.1	149.4	151.4	163.5	177.9
		35	149.5	149.0	149.0	151.1	165.4	173.5
		-----	-----	-----	-----	-----	-----	-----
		Mean	146.03	145.32	147.28	152.37	166.17	177.67
		S.D.	3.39	5.05	2.75	1.94	3.12	4.06
N	6	6	6	3	3	3		

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 4.1.1 – Absolute and relative lung weight at necropsy on day 1 - Males

Day: 1 relative to Start Date					
Group	Sex	Animal	Bodywt g	Lungs g	Lungs relative g/kg body wgt
1	m	2	212.9	1.07	5.03
		4	201.1	1.06	5.27
		6	213.2	1.05	4.92
		-----	-----	-----	-----
		Mean	209.07	1.060	5.073
		S.D.	6.90	0.010	0.179
2	m	N	3	3	3
		14	200.2	1.02	5.09
		16	216.3	1.18	5.46
		18	208.4	1.10	5.28
		-----	-----	-----	-----
		Mean	208.30	1.100	5.277
3	m	S.D.	8.05	0.080	0.185
		N	3	3	3
		26	214.7	1.19	5.54
		28	207.7	1.15	5.54
		30	215.5	1.16	5.38
		-----	-----	-----	-----
		Mean	212.63	1.167	5.487 *
		S.D.	4.29	0.021	0.092
		N	3	3	3

Statistics: One-way Analysis of Variance / Dunnett's test: \* p < 0.05

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 4.1.2 – Absolute and relative lung weight at necropsy on day 1 - Females

Day: 1 relative to Start Date					
Group	Sex	Animal	Bodywt g	Lungs g	Lungs relative g/kg body wgt
1	f	1	148.4	0.92	6.20
		3	147.8	0.89	6.02
		5	150.6	0.89	5.91
		-----	-----	-----	-----
		Mean	148.93	0.900	6.043
		S.D.	1.47	0.017	0.146
		N	3	3	3
2	f	13	154.9	0.93	6.00
		15	147.4	0.88	5.97
		17	147.0	0.87	5.92
		-----	-----	-----	-----
		Mean	149.77	0.893	5.963
		S.D.	4.45	0.032	0.040
		N	3	3	3
3	f	25	144.9	0.91	6.28
		27	150.8	0.90	5.97
		29	145.0	0.96	6.62
		-----	-----	-----	-----
		Mean	146.90	0.923	6.290
		S.D.	3.38	0.032	0.325
		N	3	3	3

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 4.2.1 – Absolute and relative lung weight at necropsy on day 14 – Males

Day: 14 relative to Start Date

Group	Sex	Animal	Bodywt	Lungs	Lungs relative
			g	g	g/kg body wgt
1	m	8	301.4	1.40	4.64
		10	277.6	1.21	4.36
		12	261.6	1.16	4.43
		-----	-----	-----	-----
		Mean	280.20	1.257	4.477
		S.D.	20.03	0.127	0.146
2	m	N	3	3	3
		20	279.1	1.17	4.19
		22	292.9	1.22	4.17
		24	281.8	1.24	4.40
		-----	-----	-----	-----
		Mean	284.60	1.210	4.253
3	m	S.D.	7.31	0.036	0.127
		N	3	3	3
		32	284.6	1.27	4.46
		34	281.9	1.24	4.40
		36	289.7	1.33	4.59
		-----	-----	-----	-----
		Mean	285.40	1.280	4.483
		S.D.	3.96	0.046	0.097
		N	3	3	3

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Study:

Table 4.2.2 – Absolute and relative lung weight at necropsy on day 14 – Females

Day: 14 relative to Start Date

Group	Sex	Animal	Bodywt	Lungs	Lungs relative
			g	g	g/kg body wgt
1	f	7	168.8	0.93	5.51
		9	176.0	0.96	5.45
		11	178.2	0.97	5.44
		-----	-----	-----	-----
		Mean	174.33	0.953	5.467
		S.D.	4.92	0.021	0.038
		N	3	3	3
2	f	19	179.2	0.99	5.52
		21	182.5	0.95	5.21
		23	176.1	0.91	5.17
		-----	-----	-----	-----
		Mean	179.27	0.950	5.300
		S.D.	3.20	0.040	0.192
		N	3	3	3
3	f	31	181.6	0.97	5.34
		33	177.9	1.02	5.73
		35	173.5	0.95	5.48
		-----	-----	-----	-----
		Mean	177.67	0.980	5.517
		S.D.	4.06	0.036	0.198
		N	3	3	3

Acute (4-hour) inhalation toxicity study with in water' in rats

Study:  
Table 5.1: Summary of macroscopic observations in animals sacrificed on day 1

		INCIDENCE OF LESIONS (NUMERIC)					
		Males			Females		
CHANGES	TREATMENT	Contr.	0.1 g/m3	1.0 g/m3	Contr.	0.1 g/m3	1.0 g/m3
LUNGS							
Petechia(e)		1					
Red area left lobe				1			
Red patches							1
THYMUS							
Uni-lateral red patches		1				1	

Study : Report Complete.

Page: 1  
Date: 11-FEB-11

Acute (4-hour) inhalation toxicity study with in water' in rats

Study:

Table 5.2: Summary of macroscopic observations in animals sacrificed on day 14

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)					
		Males			Females		
		Contr.	0.1 g/m3	1.0 g/m3	Contr.	0.1 g/m3	1.0 g/m3
LUNGS							
Petechia(e) caudal lobe				1			
Red patche(s) caudal lobe		1		1			
Red area left lobe			1			2	
Red area medial lobe		1					
THYMUS							
Red patches		1		1	1	1	

Study :

Report Complete.

Page: 1  
Date: 11-FEB-11

Acute (4-hour) inhalation toxicity study with ' in water' in rats

Study:

Table 6.1: Summary of microscopic observations in animals sacrificed on day 1

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)					
		Males			Females		
		Contr.	0.1 g/m3	1.0 g/m3	Contr.	0.1 g/m3	1.0 g/m3
LARYNX		(3)		(3)	(3)		(3)
Focal mononuclear cell infiltrate		3		2	3		2
LUNGS		(3)		(3)	(3)		(3)
Focal haemorrhage(s)		2		3	3		1
Focal alveolitis		1					
NASAL CAVITY		(3)		(3)	(3)		(3)
Focal mononuclear cell infiltrate		1					
THYMUS		(1)				(1)	
Microhaemorrhage(s)		1				1	
TRACHEA/BRONCHI		(3)		(3)	(3)		(3)
Focal mononuclear cell infiltrate		1					

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Study :

Report Complete.

Page: 1  
Date: 11-FEB-11

Acute (4-day) inhalation toxicity study with ' in water' in rats

Study:

Table 6.2: Summary of microscopic observations in animals sacrificed on day 14

CHANGES	TREATMENT	INCIDENCE OF LESIONS (NUMERIC)					
		Males			Females		
		Contr.	0.1 g/m3	1.0 g/m3	Contr.	0.1 g/m3	1.0 g/m3
LARYNX		(3)		(3)	(3)		(3)
Focal mononuclear cell infiltrate		3		2	3		2
LUNGS		(3)	(1)	(3)	(3)	(2)	(3)
Focal haemorrhage(s)		3	1	2	1	2	1
Focal alveolitis		1					
Focal accumulation of alveolar macrophages			1				
Focal Haemoglobin chrystals			1	1	1		
NASAL CAVITY		(3)		(3)	(3)		(3)
Mononuclear cell infiltrate					1		
THYMUS		(1)		(1)	(1)	(1)	
Microhaemorrhage(s)		1		1	1	1	
TRACHEA/BRONCHI		(3)		(3)	(3)		(3)
No abnormality detected		3		3	3		3

Figures in brackets represent the number of animals from which this tissue was examined microscopically

Study :

Report Complete.

## Annexes

## Annex 1 – Endorsement of GLP compliance



voedsel en waren autoriteit

### ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF  
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 27-31 October 2008 at

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity, mutagenicity, analytical and clinical chemistry, kinetics and metabolism, safety pharmacology, worker exposure and in-vitro studies.



Den Haag, 03 February 2009

  
Dr Th. Helder  
Manager GLP Compliance Monitoring Program

Food and Consumer Product Safety Authority (VWA)  
Prinses Beatrixlaan 2, 2595 AL Den Haag  
Postbus 19506, 2500 CM Den Haag, The Netherlands

This endorsement is valid for the indicated areas of expertise, currently part of

## Annex 2 – Certificate of analysis of the test material

### *Certificate of Analysis*

Material:  
Lot Number:  
Date of Analysis:

(continued on page 2)

*Certificate of Analysis*

Material:  
Lot Number:  
Date of Analysis:

(continued from page 1)

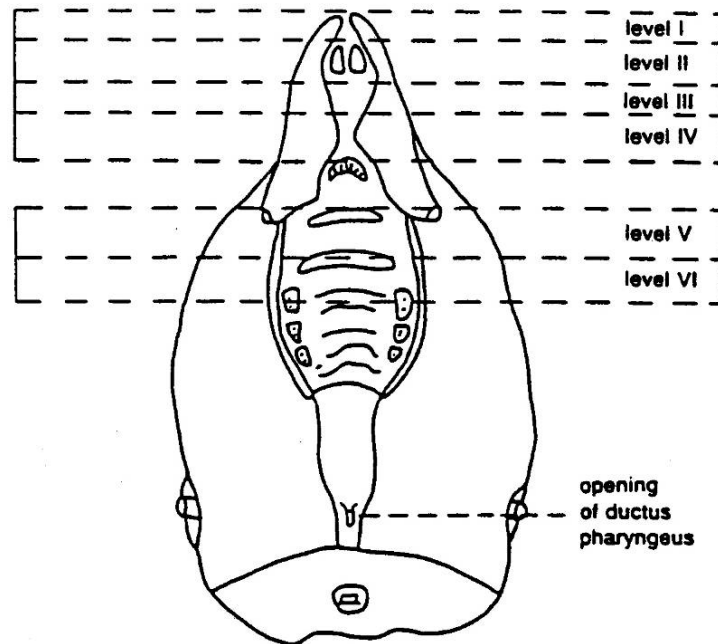
## Annex 3 – Cross reference listing

Animal -----	Group -----	Cage -----	Sex ---	Replicate -----
2	1	2	M	1
4	1	2	M	1
6	1	2	M	1
8	1	4	M	2
10	1	4	M	2
12	1	4	M	2
14	2	6	M	1
16	2	6	M	1
18	2	6	M	1
20	2	8	M	2
22	2	8	M	2
24	2	8	M	2
26	3	10	M	1
28	3	10	M	1
30	3	10	M	1
32	3	12	M	2
34	3	12	M	2
36	3	12	M	2
1	1	1	F	1
3	1	1	F	1
5	1	1	F	1
7	1	3	F	2
9	1	3	F	2
11	1	3	F	2
13	2	5	F	1
15	2	5	F	1
17	2	5	F	1
19	2	7	F	2
21	2	7	F	2
23	2	7	F	2
25	3	9	F	1
27	3	9	F	1
29	3	9	F	1
31	3	11	F	2
33	3	11	F	2
35	3	11	F	2

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## Annex 4 – Levels of cross-sections through the nasal cavity (Woutersen et al., 1994)

Ventral view of the rat hard palate region with the lower jaw removed, indicating the six standard cross-sections through the nose (I to VI; Woutersen et al., 1994).



## Annex 5 – Test site report: peer review of the pathology

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**STUDY TITLE:** Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water' in rats

**AUTHOR:**

**ANATOMIC PATHOLOGY PEER  
REVIEW REPORT COMPLETED:** April 29, 2011

**PERFORMING LABORATORY:**

**LABORATORY PROJECT ID:**

**WORK REQUEST NUMBER:**

**SERVICE CODE NUMBER:**

**SPONSOR:**

**PROJECT  
NUMBER:**

3 May, 2011

Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water' in rats

---

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

The work performed at \_\_\_\_\_ was conducted in compliance with U.S. EPA TSCA  
(40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current  
OECD Good Laboratory Practices.

Principal Investigator:

29 April 2011  
Date

Approved by  
Haskell Management:

29 April 2011  
Date

Sponsor:

29 April 2011  
Date

3 May, 2011

Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water' in rats

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### QUALITY ASSURANCE STATEMENT

Work Request Number:  
Service Code Number:  
Project Number:  
Code Number

Key inspections for the Peer Pathology review from above referenced study were completed by  
the Quality Assurance Unit of and the findings were submitted on the following  
dates:

<i>Audit Dates</i>	<i>Date Reported to:</i>			
	<i>Principal Investigator (PI)</i>	<i>PI Management</i>	<i>Study Director (SD)</i>	<i>SD Management</i>
<b><u>Protocol/Report/Records:</u></b>				
April 18, & 19, 2011	April 19, 2011	April 27, 2011	April 28, 2011	April 28, 2011

Reported by: \_\_\_\_\_

\_\_\_\_\_  
29-APR-2011  
Date

3 May, 2011

Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water<sup>1</sup> in rats

---

**CERTIFICATION**

I, the undersigned, declare that these results provide accurate data obtained from this study.

Issued by  
Principal Investigator: \_

29 April 2011  
Date

3 May, 2011

Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water<sup>1</sup> in rats

---

### SUMMARY

Gross and microscopic observations and the pathology report of this acute inhalation study with in water were peer reviewed according to guideline for conducting pathology peer review. The peer review pathologist is in essential agreement with the study pathologist regarding microscopic changes, terminology, and the overall interpretations and conclusions of the study.

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Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water<sup>1</sup> in rats

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## INTRODUCTION

This report documents the pathology peer review process that was used for review of pathology data from this study and the results of the review.

## METHODS

A peer review of respiratory tissues (nose, larynx, trachea, and lungs) from the above referenced study was completed. The review included examination of the complete set of tissues from selected controls and all high-dose animals at both the 1- and 14-day time points. In addition to the slides, summary incidence and individual animal data for gross and microscopic findings were available. A draft report stating the conclusions of the primary pathologist was available. A conduct audit was not conducted during the pathology review as this was only a peer review of pathology slides prepared by

Anatomic Pathology Peer Review Report for  
Acute (4-hour) inhalation toxicity study with  
in water' in rats

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### CONCLUSIONS

The peer review pathologist is in essential agreement with study pathologist regarding microscopic changes, terminology, and the overall interpretations and conclusions of the study.

### RECORDS AND SAMPLE STORAGE

For the work conducted at                      the anatomic pathology peer review report will be  
retained at  
or

## Appendix 1 – Individual pathology data

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Acute (4-hour) inhalation toxicity study with '  
in water' in rats

## Appendix 1.1: Individual data pathology of animals sacrificed on day 1

## Study:

Animal	Group:1	Contr.	Males
A0002	survivor killed on day 1		
MACROSCOPY : No gross lesions			
Microscopic findings			
LARYNX : Very slight focal mononuclear cell infiltrate			
LUNGS : Slight focal haemorrhage(s)			
TRACHEA/BRONCHI : Slight focal mononuclear cell infiltrate			
NO ABNORMALITIES DETECTED IN: NASAL CAVITY			
A0004	survivor killed on day 1		
Macroscopic findings			
LUNGS : Petechia(e)			
THYMUS : Uni-lateral red patches			
Microscopic findings			
LARYNX : Very slight focal mononuclear cell infiltrate			
LUNGS : Slight focal haemorrhage(s)			
Very slight focal alveolitis			
THYMUS : Microhaemorrhage(s)			
NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI			
A0006	survivor killed on day 1		
MACROSCOPY : No gross lesions			
Microscopic findings			
LARYNX : Very slight focal mononuclear cell infiltrate			
NASAL CAVITY : Very slight focal mononuclear cell infiltrate Level 5			
NO ABNORMALITIES DETECTED IN: LUNGS, TRACHEA/BRONCHI			

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Acute (4-hour) inhalation toxicity study with  
'in water' in rats

## Appendix 1.1: Individual data pathology of animals sacrificed on day 1

## Study:

Animal	Group:1	Contr.	Females
A0001	survivor killed on day 1		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		
A0003	survivor killed on day 1		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		
A0005	survivor killed on day 1		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Very slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

Appendix 1.1: Individual data pathology of animals sacrificed on day 1

Study:

Animal	Group:2	0.1g/m3	Males
B0014	survivor		
	killed on day 1		
	MACROSCOPY : No gross lesions		
B0016	survivor		
	killed on day 1		
	MACROSCOPY : No gross lesions		
B0018	survivor		
	killed on day 1		
	MACROSCOPY : No gross lesions		

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Acute (4-hour) inhalation toxicity study with  
'in water' in rats

## Appendix 1.1: Individual data pathology of animals sacrificed on day 1

Study:

Animal	Group:2	0.1g/m3	Females
B0013	survivor		
	killed on day 1		
	MACROSCOPY :	No gross lesions	
B0015	survivor		
	killed on day 1		
	Macroscopic findings		
	THYMUS :	Uni-lateral red patches	
	Microscopic findings		
	THYMUS :	Microhaemorrhage(s)	
B0017	survivor		
	killed on day 1		
	MACROSCOPY :	No gross lesions	

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

## Appendix 1.1: Individual data pathology of animals sacrificed on day 1

## Study:

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Animal	Group:3	1.0g/m3	Males
--------	---------	---------	-------

---

C0026 survivor  
killed on day 1

MACROSCOPY : No gross lesions

## Microscopic findings

LARYNX : Very slight focal mononuclear cell infiltrate  
LUNGS : Slight focal haemorrhage(s)

NO ABNORMALITIES DETECTED IN:  
NASAL CAVITY, TRACHEA/BRONCHI

C0028 survivor  
killed on day 1

## Macroscopic findings

LUNGS : Red area left lobe , 4 mm

## Microscopic findings

LUNGS : Slight focal haemorrhage(s)

NO ABNORMALITIES DETECTED IN:  
LARYNX, NASAL CAVITY, TRACHEA/BRONCHI

C0030 survivor  
killed on day 1

MACROSCOPY : No gross lesions

## Microscopic findings

LARYNX : Very slight focal mononuclear cell infiltrate  
LUNGS : Slight focal haemorrhage(s)

NO ABNORMALITIES DETECTED IN:  
NASAL CAVITY, TRACHEA/BRONCHI

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Acute (4-hour) inhalation toxicity study with  
'in water' in rats

Appendix 1.1: Individual data pathology of animals sacrificed on day 1

## Study

Animal	Group:3	1.0g/m3	Females
C0025	survivor	killed on day 1	
Macroscopic findings			
	LUNGS :	Red patches	
Microscopic findings			
	LARYNX :	Slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		
C0027	survivor	killed on day 1	
	MACROSCOPY : No gross lesions		
Microscopic findings			
	NO ABNORMALITIES DETECTED IN: LARYNX, LUNGS, NASAL CAVITY, TRACHEA/BRONCHI		
C0029	survivor	killed on day 1	
	MACROSCOPY : No gross lesions		
Microscopic findings			
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	NO ABNORMALITIES DETECTED IN: LUNGS, NASAL CAVITY, TRACHEA/BRONCHI		

\*\*\* Listing Complete \*\*\*

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Acute (4-hour) inhalation toxicity study with '  
in water' in rats

## Appendix 1.2: Individual data pathology of animals sacrificed on day 14

## Study:

Animal	Group:1	Contr.	Males
A0008	survivor killed on day 14		
MACROSCOPY : No gross lesions			
Microscopic findings			
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Very slight focal haemorrhage(s)	
		Very slight focal alveolitis	
NO ABNORMALITIES DETECTED IN:			
NASAL CAVITY, TRACHEA/BRONCHI			
A0010	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red patches caudal lobe , 2x, 1 mm	
Microscopic findings			
	LARYNX :	Slight focal mononuclear cell infiltrate	
	LUNGS :	Very slight focal haemorrhage(s)	
NO ABNORMALITIES DETECTED IN:			
NASAL CAVITY, TRACHEA/BRONCHI			
A0012	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red area medial lobe , 1 mm	
	THYMUS :	Uni-lateral red patches	
Microscopic findings			
	LARYNX :	Slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	THYMUS :	Microhaemorrhage(s)	
NO ABNORMALITIES DETECTED IN:			
NASAL CAVITY, TRACHEA/BRONCHI			

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Acute (4-hour) inhalation toxicity study with ' ' ;  
in water' in rats

## Appendix 1.2: Individual data pathology of animals sacrificed on day 14

## Study:

Animal	Group:1	Contr.	Females
A0007	survivor killed on day 14		
Macroscopic findings			
	THYMUS :	Uni-lateral red patches	
Microscopic findings			
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	NASAL CAVITY :	Very slight mononuclear cell infiltrate , level 2	
	THYMUS :	Microhaemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: LUNGS, TRACHEA/BRONCHI		
A0009	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
Microscopic findings			
	LARYNX :	Slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal Haemoglobin chrystals	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		
A0011	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
Microscopic findings			
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		

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Acute (4-hour) inhalation toxicity study with  
'in water' in rats

## Appendix 1.2: Individual data pathology of animals sacrificed on day 14

Study:

Animal	Group:2	0.1g/m3	Males
B0020	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
B0022	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red area left lobe	
Microscopic findings			
	LUNGS :	Slight focal Haemoglobin chrystals	
		Slight focal haemorrhage(s)	
		Very slight focal accumulation of alveolar macrophages	
B0024	survivor killed on day 14		
	MACROSCOPY : No gross lesions		

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Acute (4-hour) inhalation toxicity study with  
in water' in rats

## Appendix 1.2: Individual data pathology of animals sacrificed on day 14

## Study:

Animal	Group:2	0.1g/m3	Females
B0019	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red area left lobe	
Microscopic findings			
	LUNGS :	Very slight focal haemorrhage(s)	
B0021	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
B0023	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red area left lobe	
	THYMUS :	Red patches	
Microscopic findings			
	LUNGS :	Very slight focal haemorrhage(s)	
	THYMUS :	Microhaemorrhage(s)	

Acute (4-hour) inhalation toxicity study with '  
- in water' in rats

Appendix 1.2: Individual data pathology of animals sacrificed on day 14

Study:

Animal	Group:3	1.0g/m3	Males
C0032	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Red area caudal lobe	
Microscopic findings			
	LARYNX :	Slight focal mononuclear cell infiltrate	
	LUNGS :	Slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		
C0034	survivor killed on day 14		
Macroscopic findings			
	THYMUS :	Uni-lateral red patches	
Microscopic findings			
	LUNGS :	Slight focal Haemoglobin chrystals	
	THYMUS :	Microhaemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: LARYNX, NASAL CAVITY, TRACHEA/BRONCHI		
C0036	survivor killed on day 14		
Macroscopic findings			
	LUNGS :	Petechia(e) caudal lobe , 2x	
Microscopic findings			
	LARYNX :	Very slight focal mononuclear cell infiltrate	
	LUNGS :	Very slight focal haemorrhage(s)	
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		

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Acute (4-hour) inhalation toxicity study with '  
in water' in rats

## Appendix 1.2: Individual data pathology of animals sacrificed on day 14

Study:

Animal	Group:3	1.0g/m3	Females
C0031	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	LARYNX : Slight focal mononuclear cell infiltrate		
	NO ABNORMALITIES DETECTED IN: LUNGS, NASAL CAVITY, TRACHEA/BRONCHI		
C0033	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	NO ABNORMALITIES DETECTED IN: LARYNX, LUNGS, NASAL CAVITY, TRACHEA/BRONCHI		
C0035	survivor killed on day 14		
	MACROSCOPY : No gross lesions		
	Microscopic findings		
	LARYNX : Slight focal mononuclear cell infiltrate		
	LUNGS : Slight focal haemorrhage(s)		
	NO ABNORMALITIES DETECTED IN: NASAL CAVITY, TRACHEA/BRONCHI		

\*\*\* Listing Complete \*\*\*